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Award Number: W81XWH-04-1-0046

TITLE: Identify the Impact of TGF-B Signaling on the Stroma in the Progression of Prostate Cancer

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REPORT DATE: March 2005

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	March 2005	Annual (1 Mar 2004 - 28 Feb 2005)

4. TITLE AND SUBTITLE
Identify the Impact of TGF-B Signaling on the Stroma in the Progression of Prostate Cancer

5. FUNDING NUMBERS
W81XWH-04-1-0046

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 Words)

As a result of androgen ablation TGF-ß1 expression levels transiently elevate and regression of benign prostate hyperplasia as well as prostate cancer cells for the most part occur. Better understanding of prostate androgen responsiveness is critical in understanding and ultimately combating androgen-non-responsive prostate cancer. Studying the conditional TGF-ß type II receptor fibroblast knockout mouse model we developed ($Tgfbr2^{fspko}$), we found that TGF-ß signaling in the prostate stromal fibroblasts regulate both stromal and epithelial differentiation in the prostate. Notably the data dispels previous reports that TGF-ß signaling is required for myofibroblast differentiation. As proposed we attempted to develop mice that are stromally knocked out for TGF-ß signaling and express the large T antigen in the prostate epithelia, but was unsuccessful. We have however acquired techniques in our laboratory to perform tissue recombination experiments where the identical cell types (prostate stroma and epithelia) can generate prostate glands through xenografting, that display similar phenotypic characteristics of intact mice. We hope to gain permission to progress with these experiments in order to address the mechanism of stromal TGF-ß signaling impact on prostate cancer androgen responsiveness.

14. SUBJECT TERMS
Prostate Cancer

15. NUMBER OF PAGES
10

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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INTRODUCTION

The prostate epithelial and stromal compartments interact to regulate prostate development and function, in part through the tight regulation of glandular apoptosis and proliferation. These interactions are mediated by various factors that include hormones and cytokines. Specific signaling by androgens is required for prostate development and maintenance of function through the stimulation of proliferation and inhibition of apoptosis of prostatic epithelial cells (Hayward and Cunha, 2000; Montgomery et al., 2001). Cytokines such as EGF, IGF, and TGF-ß isoforms can also in-turn stimulate the expression of the androgen receptor (AR) in an androgen-independent fashion (Byrne et al., 1996; Culig et al., 1996). Often the development and progression of prostate cancer is dependent on androgens and their receptor for prostate cellular proliferation and differentiation. As a result, its inhibition has been the primary therapy for metastatic prostate cancer and much effort has been devoted to elucidating the role of the androgen receptor in prostate cancer. As a result of androgen ablation TGF-ß1 expression levels transiently elevate and regression of benign prostate hyperplasia as well as prostate cancer cells for the most part occur. Better understanding of prostate androgen responsiveness is critical since androgen-signaling antagonists are currently used in treating patients with malignant prostate cancer, benign prostate hyperplasia, and as a chemoprevention of prostate cancer in clinical trials. However populations of hormone-non-responsive cancer cells unfortunately frequently arise. The central hypothesis of this proposal is that TGF-ß signaling in the prostatic fibroblasts contributes to normal prostatic epithelial differentiation; when this signal is diminished in the case of some cancers the differentiation status of the epithelia is altered.

TGF-ß isoforms (TGF-ß1, ß2, ß3) have long been established as physiological regulators of prostate growth because of their ability to inhibit cell proliferation and mediate apoptosis (Kyprianou and Isaacs, 1989; Martikainen et al., 1990). TGF-ßs exert their effects through binding to the TGF-ß type II receptor (TßRII) and subsequent recruitment of the type I receptor (TßRI) for downstream cytoplasmic signaling through multiple parallel signaling pathways (Attisano and Wrana, 2002). TGFß plays a key role in the steroidal regulation of tissues and in the important growth regulation axis existing between androgenic signaling, smooth muscle differentiation and epithelial proliferation. The present proposal seeks to address the role of TGFß signaling in mouse prostate tissue under in vivo conditions in light of signaling pathways identified through studies in cell lines. In order to understand the role of TGF-ß in the prostate we have developed and studied mouse models, that employ the Cre-lox methodology to conditionally ablate TßRII expression in the stromal fibroblasts (Bhowmick et al., 2004) and those that express the large SV40 T antigen transgene (TAG) in the prostate epithelia (from collaborator, Dr. Robert Matusik, Vanderbilt U., TN) (Kasper et al., 1998). The proposal was based on preliminary data that ablation of TßRII in fibroblasts results in preneoplastic prostate intraepithelial neoplasia (PIN) lesions and prostate-specific TAG expression results in PIN and progression of focal adenocarcinoma (Kasper et al., 1998). We proposed to develop mice that expressed both TAG in the prostate epithelia and concomitant loss of TßRII in the stromal fibroblasts (mouse model termed TNT) to examine epithelial and stromal differentiation (Task1). Since these TNT mice were not thought not to be able to live past 8 weeks of age, we also proposed to rescue the prostatic tissues from these mice as xenografts and further study differences in cellular differentiation and androgen responsiveness (Task2).

BODY

To establish a base line for our analysis of the prostate epithelial and stromal differentiation in the prostates of the TNT mouse model we first examined the prostates of Tgfbr2^{fspko} mice (n=8). We used various relevant markers focusing on proper glandular maturation and compared it to littermate controls. Figure 1 illustrates the prostate epithelial organization is lacking with the evidence of the expression of the basal cell marker, p63, in the Tgfbr2^{fspko} prostate tissue sections in contrast to wild type controls. The prostate of Tgfbr2^{fspko} mice did not seem to differentiate completely as evidence by the absence of secretory vesicles as determined by electron microscopy (Figure 2). Interestingly, the limited prostate epithelial differentiation observed in the Tgfbr2^{fspko} accompanied limited stromal differentiation as well. In prostates from seven-week old Flox mice (control), the stromal cells expressed a combination of a-smooth muscle actin and desmin (mature smooth muscle markers) as determined by immunohistochemistry. In contrast, age-matched Tgfbr2^{fspko} prostates had elevated expression levels of a-smooth muscle actin and vimentin (myofibroblast markers), but expressed little to no desmin (n=12, Figure 3). Together, these results suggest TGF-ß signaling in the prostate stromal fibroblasts regulate both stromal and epithelial differentiation in the prostate. The data also dispels previous reports that TGF-ß signaling is required for myofibroblast differentiation. Clearly, other local growth factors have the ability to induce myofibroblast differentiation. We find that blocking TGF-ß signaling inhibits stromal maturation to smooth muscle.

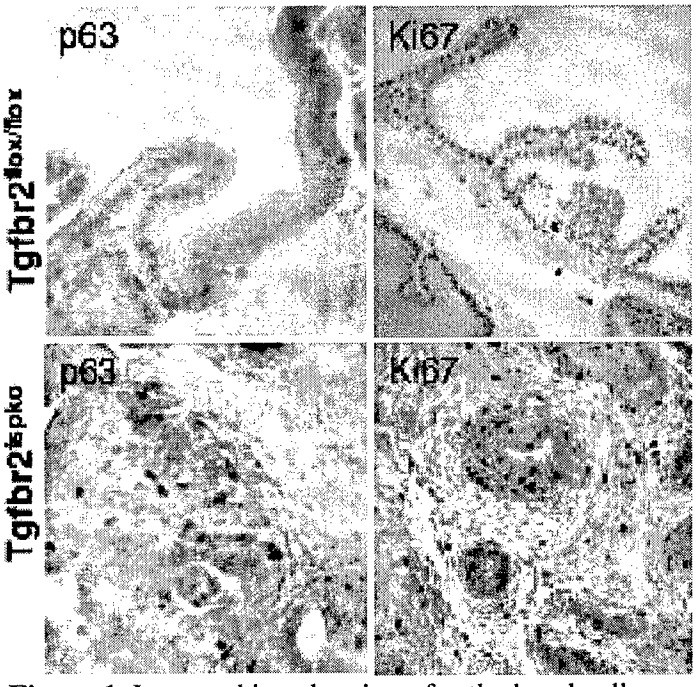

Figure 1. Immunohistochemistry for the basal cell marker, p63 and the proliferation marker, Ki67. Prostate tissues from Flox and Tgfbr2^{fspko} mice (7 weeks old).

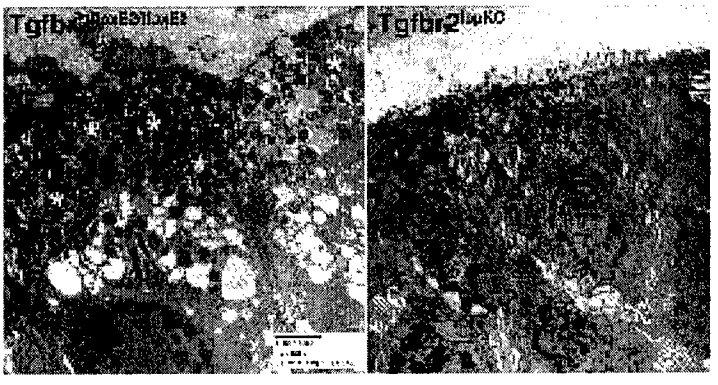

Figure 2. Electron microscopy of prostate epithelia from Flox and Tgfbr2^{fspko} mice (7 weeks old). Secretory vesicles (asterisk) were only observed in the Flox prostate glands.

Based on the approved Statement of Work (Task 1) we have attempted to develop the TNT mouse model for the past year, but have experienced little success in establishing a mouse colony that produces male TNT mice. We have discovered that the male embryos of the TNT genotype are spontaneously aborting in utero by E16. We have preliminary data now to suggest that we can achieve similar information from tissue recombination techniques developed in Gerry Cunha's laboratory (UCSF, CA) (Hayward et al., 1999; Hayward et al., 1998). Dr. Simon Hayward (collaborator, who trained with Dr. Cunha) has trained our lab members to perform the recombination procedure where prostate epithelia from either TAG or wild type mice can be combined with cultured stromal fibroblasts from Tgfbr2^{fspko} mice or control Flox mice (see Figure 4). Once the epithelial organoids and fibroblasts are embedded together in collagen I they are grafted into immuno-compromised SCID mice under the renal capsule. The renal capsule xenografting procedure was proposed in the grant as Tasks 1c and in each of the components of Task 2 since the TNT mice were anticipated to have early lethality due to forestomach cancer as we have reported in Tgfbr2^{fspko} parental mice (Bhowmick et al., 2004). However the distinction from the originally proposed Tasks and what we are hoping to have approval to perform is that the original proposal

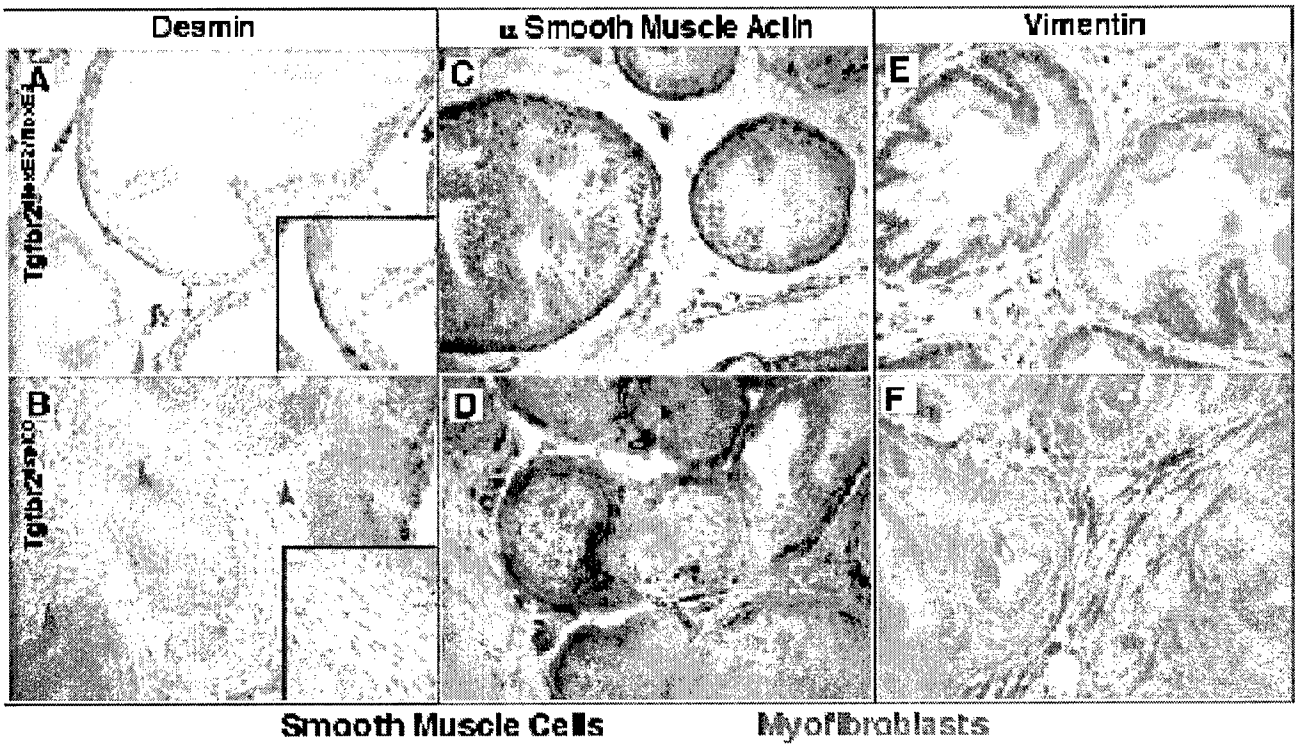

Figure 3. Immunohistochemistry for stromal differentiation marker protein expression, desmin, α smooth muscle actin, and vimentin. The prostates of Flox mice (7 week old) express desmin and α smooth muscle actin, indicitive of smooth muscle differentiation. However, prostates of age matched Tgfbr2^{fspko} mice display an expression pattern of myofibroblast cells with the positive staining for α smooth muscle actin and vimentin.

was based on the assumption that the TNT mice would be developed in a timely manner and the intact prostates would only have to be rescued in the renal capsule xenograft. Originally, the tissue rescue procedure was thought to be simpler to perform. However, in light of the difficulty we are having with developing the TNT mice and our positive experience with tissue recombination would suggest that it might be the only mechanism for us to address our hypothesis regarding the role of stromal TGF-ß signaling in cancer epithelial differentiation.

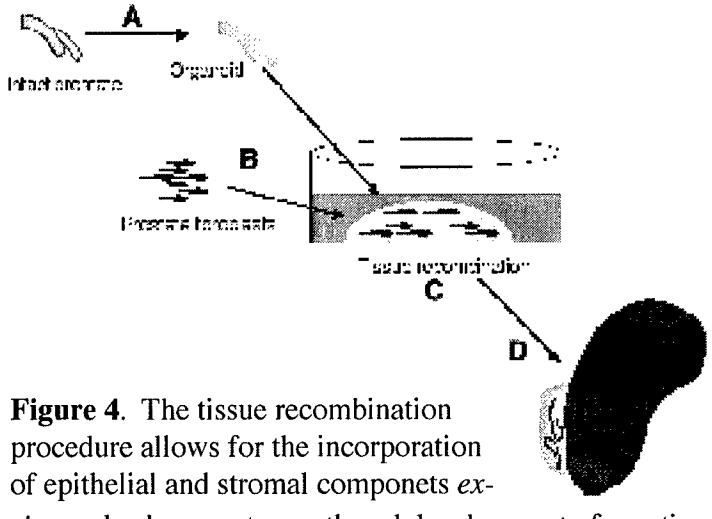

Figure 4. The tissue recombination procedure allows for the incorporation of epithelial and stromal componets *ex-vivo* and subsequent growth and develpoment of prostic cells *in vivo*. The stroma is digested from adult prostate glands (A) and combined with cultured stromal cells (B) in a collagen I gel (C). The hardened gel is then grafted under the renal capsule of SCID mice (D).

For our preliminary studies we recombined organoids from wild type mice (8-10 weeks old) and cultured stromal cells from both Flox and Tgfbr2^{fspko} mice. Since we were concerned that the cultured cells may not have the characteristics of the intact prostate stroma we determined its differentiation status through the same markers used in Figure 3. Immunofluorescence experiments indicated that the Flox stromal cells were smooth muscle and the Tgfbr2^{fspko} stromal cells were myofibroblasts, as in the intact prostates of the respective genotypes (Figure 5). The recombined tissues were engrafted for 4 weeks into 6 SCID mice, at which time 3 of the mice were castrated to assess prostate graft regression. Each of the host mice has two grafts composed of Tgfbr2^{fspko} stroma + w.t. epithelial organoid (left kidney) and Flox stroma + w.t. epithelial organoid (right kidney) for a total of 24 grafts. Figure 6 illustrates representative

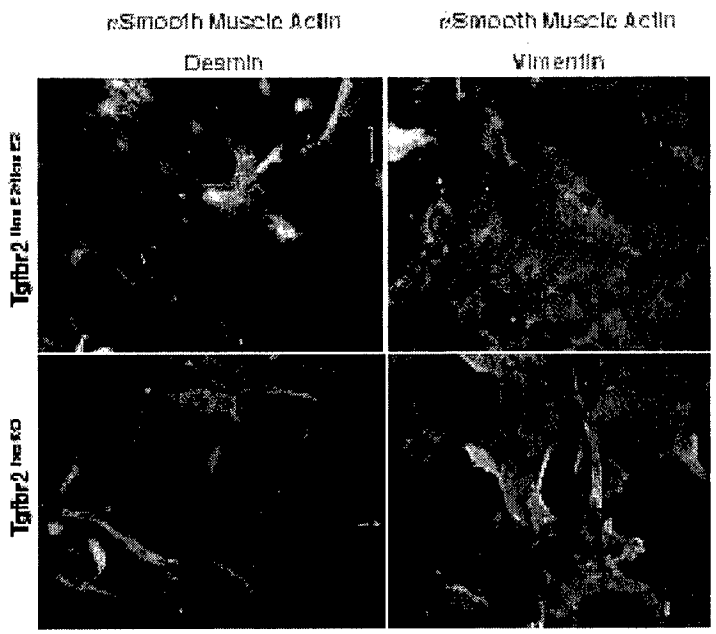

Figure 5. Cultured prostatic stromal cells display similar differentiation profile as the intact tissue (see Figure 3). The co-expression of α smooth muscle actin (green) and desmin (red) displays an yellow fluorescence. in the Flox cells. The co-expression of α smooth muscle actin (green) and vimentin (red) displays an yellow fluorescence in theTgfbr2^{fspko} cells.

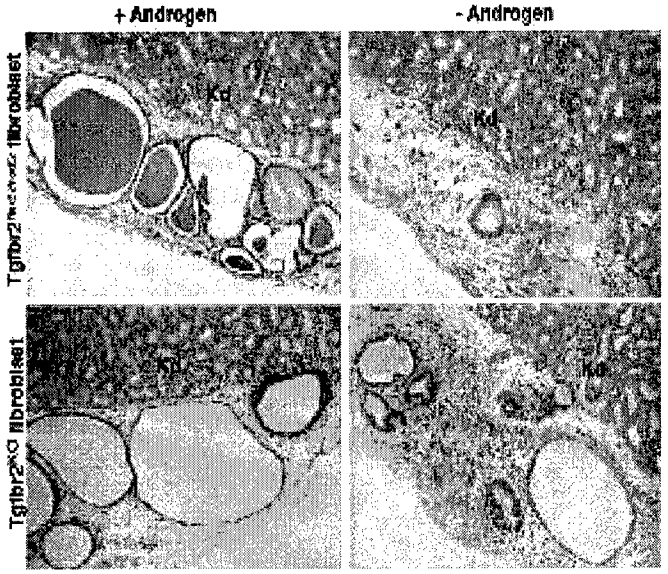

Figure 6. Prostatic tissue recombinants xenografted in SCID mice for 5 weeks were stained with H&E. The recombinant tissue components organize to form prostates the differentiate such that luminal secretions can be observed (top left). The absence of androgens results in prostatic regression in the grafts containing Flox stromal cells. Similar regression is not observed in Tgfbr2^{fspko} stromal grafts. Kd indicates kidney tissue.

hematoxylin and eosin stained paraffin sections. Comparing the recombinant prostates with Flox stroma and Tgfbr2^{fspko} stroma from in intact hosts (having androgen), we observed the prostatic secretions in the lumen of the Flox stroma grafts, however none was found in the Tgfbr2^{fspko} stroma-containing grafts. The lack of secretions in the grafts suggests a developmental deficiency in the prostatic epithelia as observed in the intact Tgfbr2^{fspko} mice (see Figure 2). The Flox stroma-containing grafts from castrated hosts (depleted androgens) showed significant regression. In contrast, those of the Tgfbr2^{fspko} stromal cells had little evidence of regression (Figure 6).

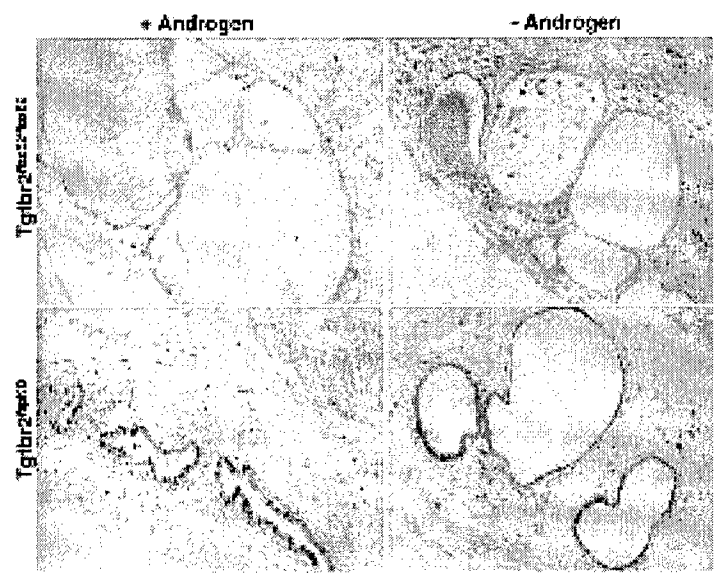

Figure 7. Immunohistochemical staining for the proliferative marker, Ki67, shows contiuned proliferation in Tgfbr2^{fspko} grafts even flowing androgen ablation.

We further stained for the proliferative marker Ki67 and found that the grafts containing Tgfbr2^{fspko} stromal cells had elevated staining compared to those with Flox stromal cells irrespective of the presence of androgens (15-fold, Figure 7). Thus we were able to recapitulate the phenotype observed in intact Tgfbr2^{fspko} mice through the tissue recombination xenografting technique. We hope to have approval to continue these studies for 8 and 30 week time courses, as in proposed in Task 2, and further incorporate epithelial cancer organoids from TAG mice in these studies. The data presented in this report is currently being written up for publication.

KEY RESEARCH ACCOMPLISHMENTS

• We showed that stromal TGF-ß signaling regulates not only prostate stromal differentiation to smooth muscle, but also dictates adjacent epithelial differentiation and organization.

• We have demonstrated that cultured prostate stromal cells when recombined with prostate epithelia can develop into prostatic grafts that engender similar phenotypes as the intact tissue.

• We provide additional in vivo data supporting the hypothesis that, stromal TGF-ß signaling can regulate prostate epithelial androgen responsiveness.

REPORTABLE OUTCOMES

Research

Manuscripts

None

Abstracts

• Bhowmick N.A. and Moses H.L. (2004) The conditional knockout of transforming growth factor-beta signaling in the prostate stroma results in prostrate intraepithelial neoplasia. American Urology Association Annual Meeting. San Francisco, CA.

• Bhowmick N.A. and Kasper, S. (2004) Prostate androgen responsiveness involves stromal transforming growth factor-beta signaling. American Urology Association Annual Meeting. San Francisco, CA.

• Placencio, V.R., Sharif-Afshar, A.-R., Moses, H.L and Bhowmick N.A. (2004) Stromal-epithelial differentiation effects in response to desmin . Society For Basic Urologic Research Meeting. Savanna, GA.

Awards received based on work supported by this grant

None

Products

CDNA construct, cell lines, and animal models developed

• Primary cell lines developed for the $Tgfbr2^{fspko}$ prostate stromal cells and Flox prostate stromal cells that maintain differentiation phenotypes of the respective intact prostates for approximately 20 passages.

CONCLUSION

The differentiation of the prostatic epithelium and stroma occur concurrently in an androgen-dependent mechanism. Further, TGF-ß signaling in the prostate stroma can dictate epithelial androgen responsiveness. To examine the impact of stromal TGF-ß signaling on the androgen responsiveness of prostate cancer cells we propose to perform tissue recombination experiments using prostate epithelial organoids from TAG mice together with Flox or $Tgfbr2^{fspko}$ stromal cells, a methodology we have shown proficiency

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